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Li, Liguang; Dechesne, Arnaud; Smets, Barth F.; Madsen, Jonas Stenlørkke; Sørensen, Søren J.

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# Permissiveness of Microbial Community from Wastewater Treatment Plant towards IncP-1 Plasmid

Liguan Li<sup>1</sup>, Arnaud Dechesne<sup>1</sup>, Barth F. Smets<sup>1,\*</sup>, Jonas Stenløkke Madsen<sup>2</sup>, Søren J. Sørensen<sup>2</sup>

<sup>1</sup> Department of Environmental Engineering, Technical University of Denmark, Denmark

<sup>2</sup> Department of Biology, University of Copenhagen

[\\*bfsm@env.dtu.dk](mailto:bfsm@env.dtu.dk)

## Abstract

Horizontal gene transfer (HGT), especially conjugal plasmid transfer, is one of the key drivers in global antibiotic resistance transmission. To predict the fate of antibiotic resistance gene (ARG), the transfer and host range of ARG carrying plasmids in relevant microbial communities needs to be understood. Wastewater treatment plants (WWTPs) are a potential conduit of ARG transfer between human intestinal and environmental bacteria, and WWTPs are being examined as potential hot spot of antibiotic resistance dissemination. In this study, a comprehensive assessment of antibiotic resistance transmission was performed in activated sludge (AS) of WWTP. Utilizing the well-established fluorescent reporter system, plasmid permissiveness in AS microbial communities were evaluated by transfer frequency using microscopic image analysis and by host range identification through combining flow-cytometry sorting and 16S rRNA gene amplicon sequencing. Under mimic sewer conditions (e.g., synthetic wastewater as growth medium), we challenged the sampled AS communities (Danish WWTP Mølleaværket, Lyngby-Taarbæk) with model plasmids from three subclades in IncP-1 compatibility group (pKJK5 ( $\epsilon$ ), pB10 ( $\beta$ -1) and RP4 ( $\alpha$ )) which were harbored by two different host strains - *Escherichia coli* MG1655 and *Pseudomonas putida* KT2440. The results showed that different donor-plasmid combinations had distinct transfer frequencies in the AS microbial communities, ranging from  $3.39 \times 10^{-5}$  to  $5.05 \times 10^{-4}$  T/R (transconjugant/recipient) (0.3 to 5 T per 10,000 R), with the most efficient transfer realized in *E. coli* (pKJK5). Unexpected broad host range across plasmid-host pairs was revealed in phylogenetic profile of transconjugant communities with total 308 exact sequence variants distributed over 13 phyla, including major group *Proteobacteria* (mainly by *Enterobacteriales* and *Pseudomonadales* in *Gammaproteobacteria*) and a few rare phyla in Gram-positive groups (e.g., *Actinobacteria* and *Firmicutes*), indicating that ‘long-distance’ transfer across phylogenies and Gram-positive/negative might be frequent under environmental conditions.